Institute Report No. 345

Acute Oral Toxicity of Trimethylolethane Trinitrate (TMETN) in ICR Mice

Larry D. Brown, LTC, VC
Yvonne C. LeTellier, BS
Conrad R. Wheeler, PhD
and
Don W. Korte, Jr., PhD, LTC, MSC

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

July 1989

Toxicology Series: 135

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

This research was conducted in compliance with the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Richard A. Kishimoto 31 July 1989

Richard A. Kishimoto (date)
COL, MSC
Acting Commander

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No.: 345	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Division of Toxicology 6b. OFFICE SYMBOL (If applicable) SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION US Army Biomedical Research and Development Laboratory
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5010
8a. NAME OF FUNDING/SPONSORING ORGANIZATION US Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62720 / PROJECT NO. A835 / TASK NO. AB / WORK UNIT ACCESSION NO. DA303913
11. TITLE (Include Security Classification) (U) Acute Oral Toxicity of Trimethylolethane Trinitrate (TMETN) in ICR Mice	
12. PERSONAL AUTHOR(S) LD Brown, YC LeTellier, CR Wheeler, and DW Korte, Jr.	
13a. TYPE OF REPORT Institute 13b. TIME COVERED FROM 6NOV84 TO 8FEB85 14. DATE OF REPORT (Year, Month, Day) July 1989 15. PAGE COUNT 58	
16. SUPPLEMENTARY NOTATION Toxicology Series No. 135	
17. COSATI CODES FIELD / GROUP / SUB-GROUP	18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) Acute Oral Toxicity, TMETN, Trimethylolethane Trinitrate, Mouse, Mammalian Toxicology, Propellant

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The acute oral toxicity of trimethylolethane trinitrate (TMETN) was determined in male and female ICR mice by using the oral gavage single-dose method. The MLD for male mice was 829.0 ± 42.5 mg/kg and for female mice was 658.4 ± 32.7 mg/kg. TMETN had a strong effect on the nervous system as indicated by the incidence of convulsions, tremors, twitching, writhing, jumping, and catalepsy. These signs were observed within 2 hours of dosing and the majority had resolved within 72 hours of dosing. Deaths were observed acutely, with 86% occurring within four hours of dosing; no deaths were observed after 24 hours. The extent of the neurotoxic component of this clinical signs profile suggests that TMETN produces pharmacological effects in addition to those routinely associated with nitrate esters. According to the classification scheme of Hodge and Steiner, these results indicate that TMETN is a slightly toxic compound.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL RICHARD A. KISHIMOTO, COL, MSC	22b TELEPHONE (Include Area Code) (415) 561-3600 22c. OFFICE SYMBOL SGRD-ULZ

DD Form 1473, JUN 86 *Previous editions are obsolete.*

ABSTRACT

The acute oral toxicity of trimethylolethane trinitrate (TMETN) was determined in male and female ICR mice by using the oral gavage single-dose method. The MLD for male mice was 829.0 ± 42.5 mg/kg and for female mice was 658.4 ± 32.7 mg/kg. TMETN had a strong effect on the nervous system as indicated by the incidence of convulsions, tremors, twitching, writhing, jumping, and catalepsy. These signs were observed within 2 hours of dosing and the majority had resolved within 72 hours of dosing. Deaths were observed acutely, with 86% occurring within four hours of dosing; no deaths were observed after 24 hours. The extent of the neurotoxic component of this clinical signs profile suggests that TMETN produces pharmacological effects in addition to those routinely associated with nitrate esters. According to the classification scheme of Hodge and Steiner, these results indicate that TMETN is a slightly toxic compound.

KEY WORDS: Acute Oral Toxicity, Trimethylolethane Trinitrate, TMETN, Mammalian Toxicology, Mouse, Propellant

PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, Maryland 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NUMBER: 84014

STUDY DIRECTOR: LTC Don W. Korte Jr, PhD, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: LTC Larry D. Brown, DVM, VC
Diplomate, American College of
Veterinary Preventive Medicine
American Board of Toxicology

CO-INVESTIGATOR: Yvonne C. LeTellier, BS

PATHOLOGIST: LTC Lance O. Lollini, DVM, VC
Diplomate, American
College of Veterinary Pathologists

DATA MANAGER: Yvonne C. LeTellier, BS

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, SOPs, raw data, analytical, stability, and purity data of the test compound, tissues, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Trimethylolethane Trinitrate (TMETN)

INCLUSIVE STUDY DATES: 6 November 1984 - 8 February 1985

OBJECTIVE:

The objective of this study was to determine the acute oral toxicity of TMETN in male and female ICR mice.

ACKNOWLEDGMENTS

SFC Charles N. Farmer, SP4 John R.G. Ryabik, SP4 James J. Fischer, SP4 Scott L. Schwebe, SGT Steven Sano, BA, and Gerald F.S. Hiatt, PhD, provided research assistance; Charlotte L. Speckman, Richard A. Spieler, and Rick Katona provided animal care; and Colleen S. Kamiyama, Julie Peacock, and Brenda V. Goce provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84014 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr. 31 July 89

DON W. KORTE, JR., PhD / DATE
LTC, MSC
Study Director

Conrad Wheeler 31 July 89

CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist

Larry D. Brown 11 Jan 89

LARRY D. BROWN, DVM / DATE
LTC, VC
Principal Investigator

Yvonne C. LeTellier 31 Jul 89

YVONNE C. LETELLIER, BS / DATE
DAC
Co-Investigator/Data Manager

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 31 July 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84014

1. This is to certify that in relation to LAIR GLP Study 84014 the following inspections were made:

29 February 1984	-	Protocol Review
14 October 1984	-	Weighing and Dosing
14 October 1984	-	Clinical Observations
23 January 1985	-	Weighing and Dosing

2. The institute report entitled "Acute Oral Toxicity of Trimethylolethane Trinitrate (TMETN) in Mice," Toxicology Series 135, was audited on 7 July 1989.

Carolyn M. Lewis

CAROLYN M. LEWIS
Diplomate, American Board of
Toxicology
Quality Assurance Auditor

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

Acute Oral Toxicity of Trimethylolethane Trinitrate (TMETN) in ICR Mice--Brown *et al.*

INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A health effects review (1) conducted for the US Army Biomedical Research and Development Laboratory (USABRDL) identified numerous gaps in the toxicology database of these compounds. Consequently, USABRDL tasked the Division of Toxicology, LAIR, to conduct an initial health effects evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, acute dermal toxicity tests in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the acute oral toxicity of trimethylolethane trinitrate (TMETN) in male and female ICR mice.

MATERIALS

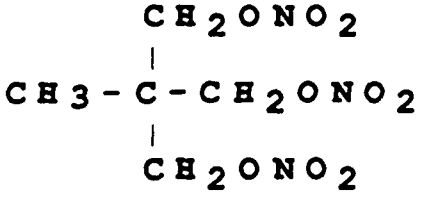
Test Substance

Chemical Name: Trimethylolethane Trinitrate (TMETN)

Chemical Abstract Service Registry No.: 3032-55-1

Toxicology Code No.: TA35

Chemical Structure:



Molecular Formula: $C_5H_9N_3O_9$

Source: Naval Ordnance Station
Indian Head, MD

Other test substance information is presented in Appendix A.

Vehicle

The vehicle for TMETN was corn oil (Lot 13F-0705, Sigma Chemical Co, St. Louis, MO).

Animal Data

ICR mice used in the study were received in separate shipments from Charles River Laboratories, Inc. (females from Portage, MA and males from Kingston, NY). Two males and 2 females were selected for quality control necropsy evaluation at receipt. The males were assigned to five dose groups and a vehicle control group. The females were assigned to five dose groups, a vehicle control group and cage control group. The animal weights on the day following receipt ranged from 22 to 32 g. Additional animal data appear in Appendix B.

Husbandry

Mice were caged individually in stainless steel wire mesh cages in racks equipped with automatic flushing dump tanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow® Diet 5002 (Ralston Purina Company, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 18.8°C to 26.7°C with a relative humidity range of 30% to 70%. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Allocation was accomplished using a computer-based stratified, weight-biased method. The Beckman TOXSYS Animal Allocation Program was used in conjunction with a Beckman TOXSYS Data Collection Terminal. Study mice were randomized into five dose groups of ten males and ten females each and vehicle control groups of five males and five females each. Five female mice were placed in a cage control group which were not dosed. Ninety-nine animals dosed with TMETN

remained on study. One male mouse was found to be a female at necropsy and was removed from the study. The animals were acclimated for 7 days before the day of dosing. During this period they were observed daily for signs of illness.

Dose Levels

The results of an approximate lethal dose (ALD) determination suggested that the MLD was between 800 and 1000 mg/kg in both sexes. Based on these data, the following test doses were selected (Table 1).

TABLE 1: TMETN Doses

Group	Dose Level mg/kg	
	Males	Females
1	vehicle control (10 ml/kg corn oil)	Cage control (no dosing)
2	631	Vehicle control (10 ml/kg corn oil)
3	794	562
4	1000	501
5	1260	631
6	1590	794
7	N/A	1000

Compound Preparation

TMETN was received as a 10% solution in ethanol. Neat TMETN was prepared by removal of the ethanol by rotoevaporation. Neat TMETN is a light brown oil. All dosing suspensions were prepared by mixing weighed volumes of neat TMETN in an appropriate volume of corn oil immediately before dosing the animals.

Chemical Analysis of TMETN and Dosing Suspensions

Periodic analysis of the ethanol solutions and neat TMETN by high presssure liquid chromatography have shown no evidence of decomposition for up to 9 weeks. Since the neat TMETN contained no impurities as determined by nuclear magnetic resonance analysis and 98% was recovered from a single peak during HPLC analysis, the neat TMETN was considered 98% pure. TMETN was shown to be stable in the corn oil vehicle for 30 days, based on storage time prior to analyses of dosing emulsions. Tests to verify concentration and to prove that homogeneous emulsions of the test compound in the vehicle could be prepared were conducted (Appendix A).

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP-OP-STX-36 (3).

The volume of dosing solution each animal received was based upon the desired dose level, and the compound concentration in solution. The dose level was increased by varying the concentration of each solution. Volumes ranged from 0.22 to 0.35 ml in the males and 0.24 to 0.32 ml in the females. The vehicle control group was given 10.0 ml/kg of corn oil. The female cage control group was untreated. Dosing was performed using the oral gavage method without animal sedation or anesthesia. Sterile disposable 1-ml B-D syringes (Bectin, Dickinson and Co., Rutherford, NJ) fitted with 20-gauge, 1-1/2 inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized. The male mice were dosed between 0849 and 1012 hours on 14 November 1984. Groups 2 and 4,5, and 6 of the female mice were dosed between 1012 and 1122 hours on 23 January 1985. After analysis of the preliminary data, Group 3 female mice were dosed between 1003 and 1016 hours on 24 January 1985 and Group 7 female mice were dosed between 1012 and 1024 hours on 25 January 1985.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages; (b) animals were removed from their cages and given a physical examination; and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Recorded observations were performed three times the first 6 hours after dosing and daily for the remainder of the 2-week test

period. A second "walk through" observation was performed daily with only significant observations recorded. Body weights were recorded weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those that survived the 14-day study period were submitted for necropsy immediately after receiving a barbiturate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. The LD_{10}, LD_{50}, and LD_{90} were derived by probit analysis using the maximum likelihood method, as described by Finney (4). The program, PROBIT, developed for the Data General Computer, Model MV8000, was used to plot the probit curve and lethal dose values.

Duration of Study

Appendix C is a historical listing of study events.

Changes/Deviations

The dosing phase of this study was accomplished according to the protocol and applicable amendments with no exceptions.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Mortality

All deaths (58) occurred within 24 hours of dosing. Thirty of the 49 males exposed to TMETN died within the first 6 hours after dosing. An additional two male animals were found dead the next morning. Twenty-six of the 50 females exposed to TMETN died within the first 24 hours after dosing. Twenty-five of these female deaths occurred within the first 6 hours after dosing and the remaining animal was found dead the next morining. Table 2 lists the compound-related deaths

by group and the percent mortality. Appendix D is a tabular presentation of cumulative mortality.

TABLE 2: Compound-Related Deaths by Group

Group	Dose Level mg/kg	Deaths/ Group	Percent Mortality
	MALE		
2	631	1/10	10
3	794	4/10	40
4	1000	8/10	80
5	1260	9/9*	100
6	1590	10/10	100
1	Vehicle Control	0/5	0
	FEMALE		
4	501	0/10	0
3	562	2/10>	20
5	631	7/10	70
6	794	8/10	80
7	1000	9/10	90
2	Vehicle Control†	0/4	20
1	Cage Control	0/5	0

* One animal (84C00535) dosed in this male group died and on necropsy was found to be a female and was therefore removed from the study.

> One animal (85C00047) was misdosed, removed from the study, and replaced with animal 85C00005.

† One animal (85C00040) was misdosed and removed from the study.

Lethal Dose Calculations

Lethal dose values were calculated by probit analysis and the equation for the probit regression line was: $Y = -28.97 + 11.64 \log X$ for males and $Y = -22.83 + 9.88 \log X$ for females, where X is the dose and Y the corresponding probit value. One misdosed and one improperly sexed animal were excluded from statistical analysis and eliminated from the study. Lethal doses calculated from the equation for the probit regression line are presented in Table 3. Figures 1 and 2 graphically present the actual data points and the regression line.

Figure 1

TMETN Dose Response Curve in Male ICR Mice

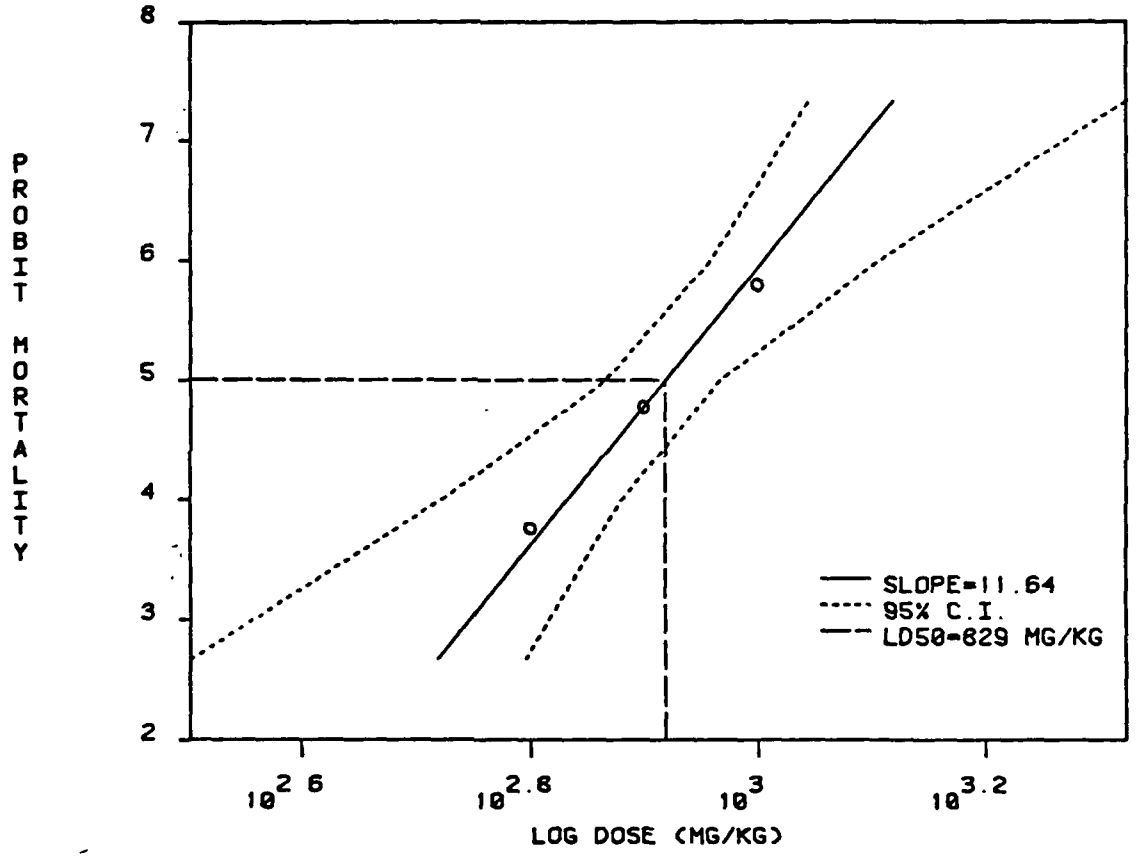


Figure 2

TMETN Dose Response Curve in Female ICR Mice

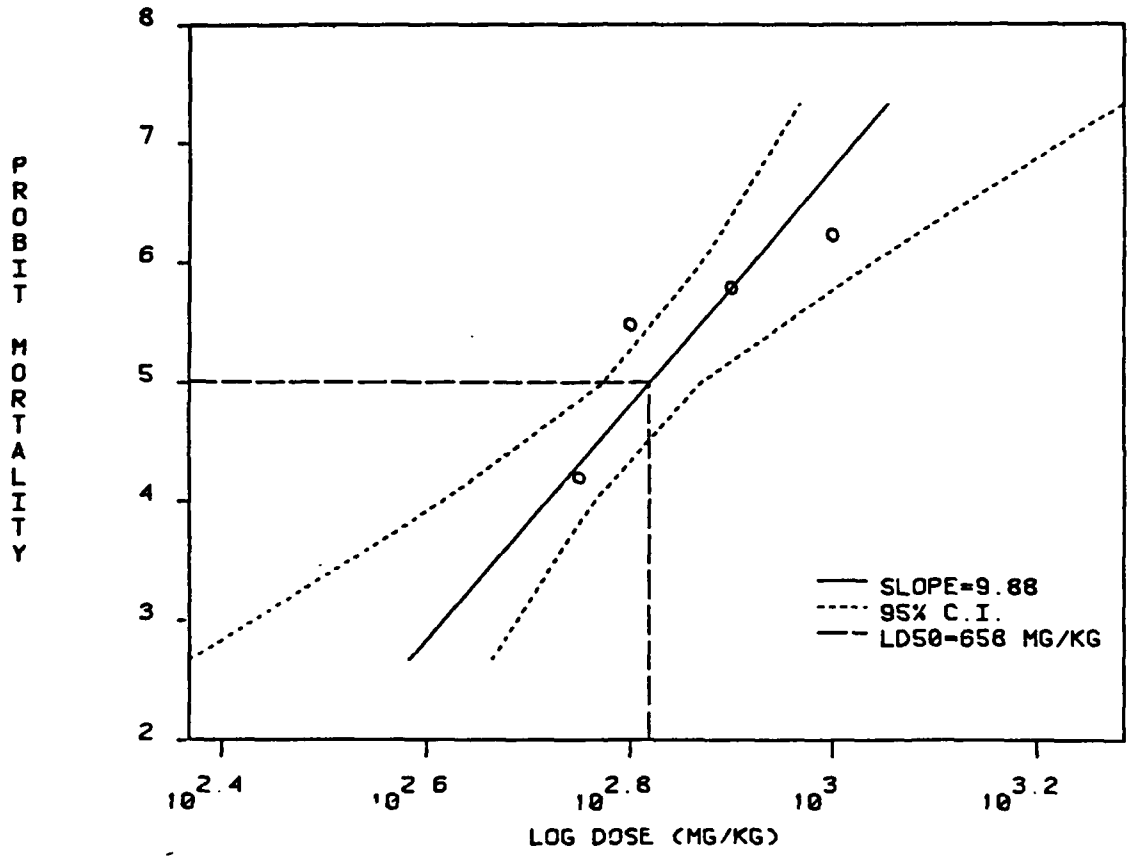


TABLE 3: Calculated Lethal Doses (MLD) of TMETN in ICR Mice

Level	Calculated Dose* (mg/kg)	95% Confidence Limits (mg/kg)
MALES		
LD10	643.3 ± 54.8	(475.4, 727.7)
LD50	829.0 ± 42.5	(734.9, 927.2)
LD90	1068.2 ± 85.6	(949.5, 1413.2)
FEMALES		
LD10	488.3 ± 40.9	(366.6, 551.7)
LD50	658.4 ± 32.7	(592.9, 740.1)
LD90	887.6 ± 81.9	(777.9, 1223.7)

* Calculated dose ± standard error.

Clinical Observations

The most frequently observed categories of clinical signs in animals administered TMETN were convulsions (68 of 99), behavioral disturbances (57 of 99 animals dosed), changes in reflex activity (39 of 99), respiratory changes (27 of 99), and hunched posture (21 of 99). Convulsions included tonic and clonic convulsions and opisthotonos. Behavioral signs included irritability, inactivity, hyperactivity, jumping, hypertonia, tremors, twitching, head pressing, vocalization, hypotonia, writhing,and catalepsy. Changes in reflex activity include depressed grasping and righting reflexes and changes in the startle reflex. Respiratory changes were tachypnea and increases in rate and depth. Although clinical signs were observed at each dose level, there was no clear dose-response relationship for severity or duration of the symptoms.

Twenty-six female mice died during the study. Of the 24 surviving female mice, 12 were normal within 24 hours and another 2 mice were normal within 48 hours. Thirty-two male mice died during the study. Of 18 surviving males, 7 were normal within 24 hours. All vehicle control and cage control animals survived until study termination at 14 days.

Table 4 contains a summary of clinical observations. Appendix E contains individual animal histories. Weight gains of survivors were not affected by dosing. Table 5 presents the mean body weights by groups. Appendix F contains individual weight tables.

Gross Pathological Observations

All study animals, whether dying acutely from TMETN or sacrificed at the end of the 14-day observation period, were examined grossly at necropsy. Few gross lesions were observed and none of these appeared clearly related to TMETN administration. The veterinary pathologist's report appears in Appendix G.

DISCUSSION

Female ICR mice were more sensitive than males to TMETN toxicity. The calculated MLD for TMETN was 829.0 ± 42.5 mg/kg in male ICR mice and 658.4 ± 32.7 mg/kg in female ICR mice. These MLD values place TMETN in the "slightly toxic" range by the system of Hodge and Sterner (5).

TMETN appeared to have a strong neurotoxic effect as convulsions were observed in 68 of 78 animals that survived to the first observation period. This is similiar to the toxicity of another nitrate ester, TEGDN, which caused acute convulsive episodes (6). There were no indications of methemoglobinemia other than increased respiration in approximately one-third of the animals. TMETN produced other signs of neurotoxicity including jumping behavior, tremors, twitching, writhing, and catalepsy. TMETN also caused the animals to exhibit hunched posture, which is an indicator of general ill health. These data suggest that the lethality due to TMETN, like the lethality of TEDGN, has a significant neurotoxic component (6), rather than being attributable to methemoglobin formation as described for nitrate esters such as PGDN (7).

Other signs of toxicity such as vocalization, irritability, inactivity, depressed grasping reflex, increased respiratory rate, hyperactivity, fecal stains, and

TABLE 4: Incidence Summary for Clinical Observations in Mice Administered TMETN

MALES

Category of Clinical Signs	Group	1	2	3	4	5	6
	(N=)	5	10	10	10	9	10
	Dose (mg/kg)	Control	631	794	1000	1260	1590
Convulsions[a]		0	6	5	4	4	4
Reflex[b]		3	0	2	3	0	1
Behavorial[c]		2	4	5	2	3	5
Respiratory[d]		3	1	2	2	3	3
Hunched Posture		0	2	3	1	1	0
Rough Coat		2	0	0	1	0	0
Miscellaneous[e]		1	2	1	0	1	0
Death without signs		0	1	2	5	4	5
Normal		1	1	0	0	0	0

FEMALES

Category of Clinical Signs	Group	1/2	3	4	5	6	7
	(N=)	9	10	10	10	10	10
	Dose (mg/kg)	Control[f]	501	562	637	794	1000
Convulsions[d]		0	9	9	9	9	9
Reflex[f]		2	7	2	7	8	9
Behavorial[c]		4	9	4	8	9	8
Respiratory[a]		0	6	2	3	2	3
Hunched Posture		0	2	1	3	6	2
Prostration		0	0	1	1	0	0
Miscellaneous[b]		1	1	0	0	0	0
Death without signs		0	0	1	1	1	1
Normal		4	0	0	0	0	0

[a]Includes tonic and clonic convulsions and opisthotonos.

[b]Includes depressed grasping and/or righting reflexes, and changes in the startle reflex.

[c]Includes irritability, inactivity, hyperactivity, jumping, hypertonia, tremors, twitching, vocalization, writhing, head pressing, and catalepsy.

[d]Includes tachypnea and changes in rate or depth.

[e]Includes feces and perianal stains.

[f]Figures represent 9 animals, 5 from cage control (Group 1) and 4 from vehicle control (Group 2).

Table 5: Mean Body Weights in Grams ± S.E.

Dose Groups (mg/kg)	Receipt	Dosing Day 0	Day 8	Termination Day 14*
		MALES		
631	25.4 ±0.6 (10)	29.5 ±0.6 (10)	31.1 ±1.3 (9)	32.6 ±1.0 (9)
794	26.1 ±0.5 (10)	29.5 ±0.4 (10)	32.0 ±0.9 (6)	33.8 ±0.9 (6)
1000	25.8 ±0.6 (10)	29.9 ±0.8 (10)	34.5 ±0.5 (2)	35.5 ±0.5 (2)
1260	25.8 ±0.6 (9)	29.1 ±0.6 (9)		
1590	26.5 ±0.7 (10)	29.9 ±0.8 (10)		
Vehicle Control	26.4 ±0.6 (5)	29.8 ±0.4 (5)	32.0 ±0.4 (5)	33.6 ±0.4 (5)
		FEMALES		
501	26.1 ±0.7 (10)	27.3 ±0.4 (10)	28.6 ±0.6 (10)	29.7 ±0.5 (10)
562	27.5 ±0.4 (10)	28.3 ±0.6 (10)	28.6 ±1.3 (8)	29.0 ±1.0 (8)
631	27.4 ±0.6 (10)	27.9 ±0.6 (10)	29.3 ±1.3 (3)	30.0 ±1.7 (3)
794	27.1 ±0.7 (10)	27.5 ±0.7 (10)	28.5 ±1.5 (2)	29.5 ±1.5 (2)
1000	26.8 ±0.6 (10)	28.0 ±0.6 (10)	26.0 (1)	26.0 (1)
Vehicle Control	26.4 ±0.6 (5)	27.2 ±0.2 (5)	28.0 ±0.7 (4)	28.0 ±0.7 (4)
Cage Control	27.0 ±0.4 (5)	29.0 ±0.4 (5)	28.6 ±0.5 (5)	28.6 ±0.6 (5)

* Weights after fast.

rough coat were observed in the control as well as treated animals and thus could not be attributed to TEGDN.

No gross lesions were observed in the animals which could account for the toxicity observed. This may reflect the short time between dosing and death. All mice deaths occurred within 24 hours; 86 percent of the deaths occurred within 4 hours.

CONCLUSION

TMETN is a nitrate ester with significant neurotoxicity. Female mice were more sensitive to TMETN than males. Calculated MLD values were 829.0 $\pm$ 42.5 mg/kg in male ICR mice and 658.4 $\pm$ 32.7 mg/kg in female ICR mice.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC NO. ADA 127846.

2. Enviromental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, oral toxicity. In: Health effects test guidelines. Washington, DC; Environmental Protection Agency, August 1982; EPA 560/6-82-001.

3. Acute oral toxicity study (ALD and LD_{50}). LAIR Standard Operating Procedure OP-STX-36, Presidio of San Francisco, California: Letterman Army Institute of Research, 15 June 1984.

4. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press, 1971:20-80.

5. Hodge HC, Sterner JH. Tabulation of toxicity classes. Amer Ind Hyg Assoc Q 1943; 10:93-96.

6. Andersen ME, Mehl, RG. A comparison of the toxicology of triethylene glycol dinitrate and propylene glycol dinitrate. Amer Ind Hyg Assoc J 1973; 34:526-532.

7. Jones RA, Strickland JA, Siegal J. Toxicity of propylene glycol 1,2-dinitrate on experimental animals. Toxicol Appl Pharmacol 1972; 22:128-137.

Appendix A: CHEMICAL DATA

Chemical Name: 1,3-Propanediol, 2-methyl-2 [(nitroxy)methyl]-dinitrate (ester)

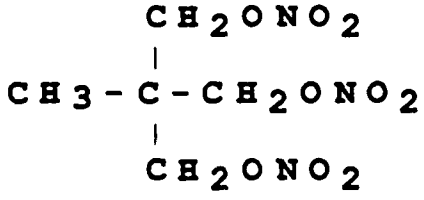
Other Names: 1,3-Propanediol-2-(hydroxymethyl)-2-methyl-, trinitrate; 1,1,1-trimethylolethane trinitrate (TMETN), metriol trinitrate (MTN); nitropentaglycerin

Lot Number: 53-84A

Chemical Abstracts Service Registry No.: 3032-55-1

LAIR Code No.: TA35

Structural Formula:



Molecular Formula: $C_5H_9N_3O_9$

Molecular Weight: 255.15

Physical State: Light brown oil

Melting Point: -3° [1,2]

Compound Density: 1.47 g/cm [1,2]

Source: Naval Ordnance Station, Indian Head, MD, 20640

---

[1] Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846, p 17.

[2] Lindner V. Properties of explosive aliphatic nitrate esters. Table 5. In: Grayson M., exec. ed. Kirk-Othmer Encyclopedia of Chemical Technology. Volume 9. 3rd ed. New York: John Wiley and Sons, Inc., 1980:573.

Appendix A (cont.): CHEMICAL DATA

Analytical Data: Ultra-violet (UV) spectra were obtained using a Hitachi 110-A Spectrophotometer (Hitachi Instruments, Inc., Mountain View, CA), infra-red spectra (IR) were obtained with a Perkin-Elmer Model 457 Infra-red Spectrophotometer (Perkin-Elmer, Norwalk, CT) and nuclear magnetic resonance (NMR) spectra were recorded on a Varian FT-80 NMR (Varian, Palo Alto, CA) using tetramethylsilane as an internal standard. Chromatographic analysis was performed using a 1090B HPLC with diode array detector (Hewlett-Packard, Santa Clara, CA) and a Brownlee RP-18 Spheri-5 Column, 4.6 x 250 mm (Brownlee Labs, Inc., Santa Clara, CA). The following conditions were employed for the HPLC assay: solvent system, 70% methanol, 30% water; flow rate, 0.9 ml/min; detector wavelength, 215 nm; oven temperature, 50°C.

UV Spectrum: For UV analysis TMETN was dissolved in acetonitrile. UV absorbance begins at approximately 240 nm and increases with decreasing wavelength.[3] No absorption peak was observed. IR (KBr windows): 2900, 1645 (asymmetric stretch of NO group), 1470, 1375, 1280 (symmetric stretch of NO_2 group), 990, 860, and 755 cm.[4] ^1H NMR ($CDCl_3$, 80 MHz): d 1.22 (S, 3H, CH_3), 4.44 (S, 6H, $-CH_2-$).[5] TMETN subjected to HPLC analysis eluted as two peaks with retention times of 5.5-5.6 and 12.5 min.[6] Based on integration of peak areas, the first peak represented 98% of the sample. The second peak was not identified. No decomposition of TMETN was detected by HPLC after storage of TMETN (neat or in ethanol) for a period of nine weeks.[7]

---

[3] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, p 51. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[4] Wheeler, CR. Nitrocellulose-Nitroguanidine Project .. oratory Notebook #84-05-010.2, p 67. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[5] *Ibid.*, p 68.

[6] Wheeler, CW. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, p 72-75. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[7] Wheeler, CW. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p 34. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

ANALYSIS OF TMETN DOSING FORMULATIONS

INTRODUCTION

Emulsions of trimethylolethane trinitrate (TMETN) in corn oil were prepared by shaking or stirring mixtures of the two components. The emulsions were subsequently used for dosing animals in the GLP studies 84013 (acute oral toxicity in rats) and 84014 (acute oral toxicity in mice). After dosing, the remainder of the emulsion was stored at 4°C for analysis. Determination of the TMETN concentration was accomplished by reverse-phase high pressure liquid chromatography.

MATERIALS

Chromatographic analysis was performed using a Hewlett-Packard 1090 high pressure liquid chromatography (HPLC) system with diode array detector (Hewlett-Packard, Palo Alto, CA). Separations were obtained on a Brownlee RP-18 column (4.6 x 250 mm, Brownlee Labs, Inc., Santa Clara, CA). HPLC grade acetonitrile and water were obtained from the J. T. Baker Chemical Co., Phillipsburg, NJ.

METHODS

Analysis of TMETN solutions was accomplished under the following HPLC conditions: solvent 70% acetonitrile-30% water; solvent flow, 0.9 ml/min; injections volume, 10 µL; detector wavelength, 205 nm.[8] The HPLC mobile phase was used to prepare standards as well as to extract the TMETN/corn oil mixtures. Standards were prepared by weighing TMETN on aluminium foil (0.5 mm squares) using a microbalance. The weigh boats containing TMETN were added to volumetric flasks. The flasks were filled to volume with the HPLC solvent and the contents were mixed well by shaking. The concentration of the standards ranged from 52 to 494 or 511 µg/ml and a set of 10 standards covering this range was analyzed both before and after each set of samples (diluted dosing emulsions).

---

[8] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p. 69-74. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

To extract the dosing preparations the TMETN/corn oil mixtures were removed from the refrigerator and warmed to room temperature. After rapidly stirring each sample for a minimum of five minutes, an aliquot of approximately one ml was removed and transferred to a tared volumetric flask. The weight of each aliquot transferred was recorded and the flask filled to volume. A second dilution was required prior to analysis by HPLC.

To determine if the emulsions of TMETN in corn oil prepared for dosing were homogenous, a series of emulsions were prepared with TMETN concentrations spanning the range of concentrations employed in the dosing preparations. Four emulsions containing 50, 200, 400 and 800 mg of TMETN per ml were prepared in 20 ml scintillation vials. After stirring with a magnetic stir bar for at least 5 minutes, aliquots from the top, middle, and bottom of the emulsions were removed and transferred to tared 25 ml volumetric flasks. The exact weight of the aliquot was recorded and the flask filled to volume. One ml of this solution was transferred to a second volumetric flask for a further dilution prior to HPLC analysis.

RESULTS

Under the conditions of the analysis, TMETN eluted with a retention time of 5.3 min. A plot of TMETN concentration versus peak area was linear within the range of concentrations (5.2 to 511 µg/ml) employed as standards. Consecutive analyses (n = 10) were performed with standards containing 52, 257, and 494 µg TMETN/ml. The coefficient of variation for each set of peak area values was 0.37%, 0.16%, and 0.6%, respectively.[8] Standards were analyzed both before and after the analysis of samples prepared from dosing emulsions. The differences in peak areas between corresponding standards run before and after was less than 1%. In addition, the difference among standards analyzed several days apart was also less than 1%.

Extraction of the dosing emulsions with 70% acetonitrile-30% water resulted in quantitative recovery of TMETN with no peaks in the chromatogram from corn oil. The results for the determination of homogeneity are presented in Table 1. The deviation of individual values from the mean of each set of three samples (top, middle, bottom) did not exceed 0.8% for any emulsion prepared.

---

[8] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p. 69-74. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

DISCUSSION

The linearity of the calibration plot and the precision of the assay within and between days indicate the assay is a valid method to quantitate TMETN. The data in Table 1 demonstrate that the dispersion of TMETN in corn oil provides a homogenous emulsion over a range of 50 to 800 mg/ml. Since the dosing preparations were prepared in an identical manner they were, by implication, homogenous.

The data from the analysis of the dosing emulsions is presented in Table 2. The concentration of TMETN determined by analysis was very close to the target value for almost every suspension prepared. In only one case (study 84014, 159.0 mg TMETN/ml) did the actual concentration come close to a 10% deviation from the target concentration.

CALCULATIONS

A series of standards were analyzed before and after the samples (diluted dosing emulsions) for each study. The two peak area values for each standard solution were averaged and linear least squares regression performed on the concentration versus peak area data. This provided the equation for the best fitting line in the form of Equation 1 in which:

Equation 1 $$y \text{ (peak area)} = mx + b$$

m is the slope, x is the concentration (µg/ml) and b is the intercept. The concentration of TMETN in the final dilution was calculated by substituting for the y the peak area obtained from HPLC analysis and solving for x.

The total amount of TMETN (mg) in the sample analyzed was calculated as shown in Equation 2:

Equation 2 $$\text{Total TMETN (mg)} = \frac{X \times \text{(dilution factor)}}{10^3 \text{ µg/mg}} = Y$$

The volume corresponding to the weight of TMETN calculated above was determined by dividing by the density of TMETN (Equation 3):

Equation 3. $$\text{Total TMETN (ml)} = \frac{\text{Total TMETN (mg)}}{1470 \text{ mg/ml}} = \frac{Y}{1470} = Z$$

Appendix A (cont.): CHEMICAL DATA

The contribution by corn oil to the volume of the original aliquot of emulsion removed for analysis was calculated as follows (Equation 4):

$$\text{Volume of corn oil} = \frac{\text{weight of aliquot removed for analysis} - \text{weight of TMETN}}{\text{Density of corn oil}} = \frac{\text{wgt(mg) of aliquot} - Y}{918\ \text{mg/ml}} = V$$

The concentration of TMETN could be determined as follows (Equation 5):

$$\text{Conc. of TMETN (mg/ml)} = \frac{\text{mg TMETN}}{\text{total volume of aliquot removed for analysis}} = \frac{\text{mg TMETN}}{\text{ml TMETN + corn oil}} = \frac{Y}{Z + V}$$

TABLE 1

Assessment of homogeneity for TMETN/corn oil emulsions. Aliquots of approximately one ml were withdrawn from the top (T), middle (M), and bottom (B) of emulsions prepared to represent the range of TMETN concentrations [TMETN] employed in dosing. Equation for line obtained by linear least squares regression: Y = 0.0653X + 0.0380 (R = 0.9996).[9]

Target [TMETN] (mg/ml)	Site of Sampling	[TMETN] Determined by Analysis (mg/ml)	Mean [TMETN] (mg/ml)	Deviation from Mean (%)
	T	49.9		0.0
50	M	49.8	49.9	0.2
	B	49.9		0.0
	T	200.4		0.0
200	M	200.0	200.4	0.2
	B	200.7		0.2
	T	404.3		0.1
400	M	404.9	403.0	0.5
	B	399.8		0.8
	T	797.1		0.3
800	M	803.2	799.4	0.5
	B	798.0		0.2

[9] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p. 10-14, 26-35. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

TABLE 2

Concentration of TMETN [TMETN] in dosing formulations prepared for GLP studies 84013 and 84014.

Study #	Target [TMETN] (mg/ml)	Date Prepared	Date Analyzed	[TMETN] determined by analysis (mg/ml)	% target [TMETN]
84013[10]	400.0	19 Oct 84	21 Feb 85	405.3	101.3
	800.0	19 Oct 84	21 Feb 85	795.3	99.4
	77.5	23 Oct 84	21 Feb 85	80.7	104.1
	100.0	23 Oct 84	21 Feb 85	96.5	96.5
	129.0	23 Oct 84	21 Feb 85	128.0	99.2
	167.0	23 Oct 84	21 Feb 85	166.6	99.8
	215.0	23 Oct 84	21 Feb 85	220.9	102.7
	278.0	23 Oct 84	21 Feb 85	273.6	98.4
	60.0	25 Oct 84	21 Feb 85	59.6	99.3
	100.0	25 Oct 84	21 Feb 85	100.8	100.8
	129.0	25 Oct 84	21 Feb 85	128.1	99.3
	360.0	25 Oct 84	21 Feb 85	373.5	103.8
84014[11]	100.0	9 Nov 84	25 Feb 85	97.2	97.2
	63.1	13 Nov 84	25 Feb 85	64.9	102.8
	79.4	13 Nov 84	25 Feb 85	79.4	100.0
	100.0	13 Nov 84	25 Feb 85	96.2	96.2
	126.0	13 Nov 84	25 Feb 85	130.5	103.6
	159.0	13 Nov 84	25 Feb 85	174.2	109.6
	39.8	23 Jan 85	25 Feb 85	38.4	96.5
	50.1	23 Jan 85	25 Feb 85	49.6	99.0
	79.4	23 Jan 85	25 Feb 85	78.9	99.4
	100.0	23 Jan 85	25 Feb 85	98.8	98.8
	56.2	24 Jan 85	25 Feb 85	55.5	98.8

[10] Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p. 6-14, 26-35. Letterman Army Institute of Research, Presidio of San Francisco, CA. The equation obtained for the standard curve was $y = 0.0653X + 0.0380$ ($R = 0.9996$).

[11] *Ibid.* p. 15-17, 26-35. The equation obtained for the standard curve was $y = 0.0639X + 0.2973$ ($r = 0.9998$).

Appendix B: ANIMAL DATA

Species: *Mus musculus*

Strain: CD-1 (ICR)

Source: Charles River Laboratories, Inc.
Kingston, NY, and Portage, MA

Sex: Male and female

Date of birth: 21 September 1984 (males)
13 November 1984 (females)

Method of randomization: Weight bias, stratified animal allocation (TOXSYS® Animal Allocation Program, SOP OP-ISG-24)

Animals in each group: 10 males and 10 females initially
5 each for control groups

Condition of animals at start of study: Normal

Body weight range at dosing: 22-35 g

Identification procedures: Cervical tag

Pretest conditioning: Quarantine/acclimation 7-13 November 1984 (males) and 16-22 January 1985 (females).

Justification: The laboratory mouse has proven to be a sensitive and reliable animal model for lethal dose determination studies.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
6 Nov 84	Fifty-eight male ICR mice were recieved, checked for physical condition, and individually caged.
7 Nov 84	Animals were weighed, sexed, and tagged.
8 Nov 84	Two male mice were submitted for necropsy quality control.
7-13 Nov 84	Animals were observed daily.
9 Nov 84	ALD animals were weighed, dosed, and observed.
13 Nov 84	Animals were weighed and randomized into dose groups.
14 Nov 84	Group 1-6 male animals were fasted 4 hours, weighed, dosed, and observed at 2, 4, and 6 hours after dosing.
15-27 Nov 84	All animals were observed daily in a.m. and p.m.
21 Nov 84	Animals were weighed.
28 Nov 84	Food was removed at 0600 hours. All surviving animals were weighed, observed, and submitted for necropsy.
15 Jan 85	Sixty-four female ICR mice were received, sexed, observed for illness, and individually caged.
16 Jan 85	Animals were weighed and tagged. Two female mice submitted for quality control necropsy.
16-22 Jan 85	Animals were observed daily.
21 Jan 85	Animals were weighed and randomized into dose groups.

Appendix C (cont.): HISTORICAL LISTING OF STUDY EVENTS

Date	Event
23 Jan 85	Group 1, 2, 4-6 female animals were fasted 4 hours, weighed, dosed, and observed at 1, 2, and 4 hours after dosing. Groups 3 & 7 were reserved for "up or down" dose level modifications as required.
24 Jan 85	Group 3 was fasted 4 hours, weighed, dosed, and observed 3 times after dosing.
25 Jan 85	Group 7 was fasted 4 hours, weighed, dosed, and observed 3 times after dosing.
24 Jan-6 Feb 85	Group 1,2,4-6 animals were observed daily for clinical signs in a.m. and p.m..
25 Jan-7 Feb 85	Group 3 animals were observed daily for clinical signs in a.m. and p.m.
26 Jan-8 Feb 85	Group 7 animals were observed daily for clinical signs in a.m. and p.m.
31 Jan-2 Feb 85	Animals were weighed.
6-8 Feb 85	Food was removed at 0600 hours. All surviving animals were weighed, examined, and submitted for necropsy.

Appendix D: CUMULATIVE MORTALITY DATA (deaths/group) (10 Animals Per Group)

Dose Level mg/kg	Time After Dosing: Hours 1	Hours 2	Hours 4	Days 1	Days 2	Days 3	Days 4	Days 5-14
MALES								
631	0	0	1	1	1	1	1	1
794	0	2	3	4	4	4	4	4
1000	4	5	8	8	8	8	8	8
1260*	1	6	7	9	9	9	9	9
1590	5	8	9	10	10	10	10	10
Controls†	0	0	0	0	0	0	0	0
FEMALES								
501	0	0	0	0	0	0	0	0
562>	1	2	2	2	2	2	2	2
631	2	6	6	7	7	7	7	7
794	3	3	6	8	8	8	8	8
1000	3	6	8	9	9	9	9	9
Controls<	0	0	0	0	0	0	0	0
Total	19	38	50	58	58	58	58	58

* Nine animals.
† Five vehicle control animals.
> Includes the animal that replaced a misdosed animal.
< Nine animals. Includes 5 cage control and 4 vehicle control animals.

APPENDIX E: INDIVIDUAL ANIMAL HISTORIES

MALE: 631 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00507	Opisthotonos	Nov. 14	Moderate
84C00512	Inactive	Nov. 15-19	Slight
84C00519	Opisthotonos	Nov. 14	Marked
	Hunched posture	Nov. 14	Slight
	Incr. Respiration Depth	Nov. 14	Slight
	Inactive	Nov. 14	Moderate
	Hypotonia	Nov. 14	Slight
	Stain, Yellow, Perianal	Nov. 21	Slight
84C00520	Opisthotonos	Nov. 14	Moderate
	Irritable	Nov. 17	Slight
84C00527	Corneal Opacity, Left Eye	Nov. 14-28	Marked
84C00536	Opisthotonos	Nov. 14	Marked
	Stain, Yellow, Perianal	Nov. 14,18-19	Slight
84C00552	Normal	N/A	N/A
84C00559	Opisthotonos	Nov. 14	Moderate
84C00560	Opisthotonos	Nov. 14	Moderate
	Inactive	Nov. 14	Slight
	Hunched Posture	Nov. 14	Slight
84C00561	Death	Nov. 14	2.3 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 794 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00506	Opisthotonos	Nov. 14	Marked
	Depr. Grasping Reflex	Nov. 14	Slight
84C00518	Opisthotonos	Nov. 14	Marked
	Tremors	Nov. 14	Moderate
	Incr. Respiration Rate	Nov. 14-21	Moderate
	Twitching	Nov. 14	Slight
	Hypertonia	Nov. 14	Moderate
	Incr. Respiration Depth	Nov. 14	Slight
84C00522	Hunched Posture	Nov. 14	Slight
	Opisthotonos	Nov. 14	Marked
	Death	Nov. 14	4.8 h
84C00523	Incr. Respiration Rate	Nov. 14	Moderate
	Hyperactive	Nov. 14	Slight
	Hunched Posture	Nov. 14	Slight
	Stain, Yellow, Perianal	Nov. 17	Slight
84C00525	Death	Nov. 14	1.1 h
84C00541	Incr. Startle Reflex	Nov. 14	Marked
	Depr. Grasping Reflex	Nov. 14	Marked
	Jumping	Nov. 14	Slight
	Hunched Posture	Nov. 14	Slight
	Death	Nov. 14	3.5 h
84C00542	Opisthotonos	Nov. 14	Slight
84C00544	Irritable	Nov. 14,17-20	Slight
84C00562	Irritable	Nov. 14	Slight
	Opisthotonos	Nov. 14	Slight
84C00563	Death	Nov. 14	2.0 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1000 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00511	Death	Nov. 14	0.8 h
84C00530	Depr. Grasping Reflex	Nov. 14	Slight
	Death	Nov. 14	3.0 h
84C00533	Death	Nov. 14	0.9 h
84C00537	Opisthotonos	Nov. 14	Marked
	Tremors	Nov. 14	Moderate
	Death	Nov. 14	2.9 h
84C00546	Death	Nov. 14	0.9 h
84C00548	Death	Nov. 14	1.5 h
84C00551	Opisthotonos	Nov. 14	Marked
	Hunched Posture	Nov. 14	Slight
	Depr. Grasping Reflex	Nov. 15-20	Slight
	Rough Coat	Nov. 20	Slight
	Incr. Respiration Rate	Nov. 20	Slight
84C00553	Opisthotonos	Nov. 14	Marked
	Tremors	Nov. 14	Moderate
	Depr. Grasping Reflex	Nov. 14	Slight
	Incr. Respiration Rate	Nov. 14	Moderate
	Death	Nov. 14	2.6 h
84C00556	Death	Nov. 14	0.9 h
84C00558	Opisthotonos	Nov. 14	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1260 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00514	Death	Nov. 14	1.0 h
84C00531	Tremors	Nov. 14	Moderate
	Twitching	Nov. 14	Marked
	Head Pressing	Nov. 14	Marked
	Death	Nov. 14	2.1 h
84C00534	Death	Nov. 14	1.4 h
84C00535	Removed from study	N/A	N/A
84C00540	Opisthotonos	Nov. 14	Marked
	Stain, Yellow, Perianal	Nov. 14	Moderate
	Incr. Respiration Rate	Nov. 14	Moderate
	Death	Nov. 15	21.6 h
84C00543	Incr. Respiration Rate	Nov. 14	Marked
	Tremors	Nov. 14	Slight
	Opisthotonos	Nov. 14	Marked
	Inactive	Nov. 14	Slight
	Death	Nov. 14	2.0 h
84C00547	Opisthotonos	Nov. 14	Marked
	Death	Nov. 14	2.0 h
84C00550	Death	Nov. 14	1.2 h
84C00555	Death	Nov. 14	1.5 h
84C00557	Incr. Respiration Rate	Nov. 14	Slight
	Opisthotonos	Nov. 14	Moderate
	Hunched Posture	Nov. 14	Slight
	Inactive	Nov. 14	Slight
	Death	Nov. 15	21.4 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1590 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00513	Incr. Respiration Rate	Nov. 14	Moderate
	Tremors	Nov. 14	Slight
	Inactive	Nov. 14	Slight
	Death	Nov. 14	1.8 h
84C00515	Incr. Respiration Rate	Nov. 14	Slight
	Twitching	Nov. 14	Slight
	Opisthotonos	Nov. 14	Marked
	Death	Nov. 14	4.0 h
84C00517	Opisthotonos	Nov. 14	Marked
	Tremors	Nov. 14	Moderate
	Death	Nov. 14	2.0 h
84C00521	Death	Nov. 14	0.9 h
84C00526	Hypotonia	Nov. 14	Moderate
	Depr. Grasping Reflex	Nov. 14	Moderate
	Vocalization	Nov. 14	Moderate
	Irritable	Nov. 14	Slight
	Opisthotonos	Nov. 14	Moderate
	Death	Nov. 14	5.2 h
84C00529	Death	Nov. 14	0.8 h
84C00538	Opisthotonos	Nov. 14	Marked
	Inactive	Nov. 14	Slight
	Incr. Respiration Depth	Nov. 14	Slight
	Incr. Respiration Rate	Nov. 14	Moderate
	Death	Nov. 14	2.0 h
84C00539	Death	Nov. 14	0.9 h
84C00545	Death	Nov. 14	0.8 h
84C00554	Death	Nov. 14	0.9 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00508	Normal	N/A	N/A
84C00510	Depr. Grasping Reflex	Nov. 14,15	Moderate
	Incr. Respiration Rate	Nov. 14	Moderate
84C00516	Depr. Grasping Reflex	Nov. 14	Moderate
	Rough Coat	Nov. 20,21	Slight
	Incr. Respiration Rate	Nov. 20,21	Slight
84C00524	Irritable	Nov. 14	Slight
	Hyperactive	Nov. 14	Slight
84C00532	Depr. Grasping Reflex	Nov. 14	Slight
	Incr. Respiration Rate	Nov. 14,20,21	Moderate
	Inactive	Nov. 15	Slight
	Rough Coat	Nov. 16-28	Slight
	Stain, Yellow, Perianal	Nov. 17-28	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 501 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00003	Vocalization	Jan. 23	Slight
	Irritable	Jan. 23	Moderate
	Depr. Grasping Reflex	Jan. 23	Slight
85C00007	Depr. Grasping Reflex	Jan. 23	Slight
	Clonic Convulsion	Jan. 23	Moderate
85C00009	Inactive	Jan. 23	Slight
	Depr. Grasping Reflex	Jan. 23	Slight
	Clonic Convulsion	Jan. 23	Slight
	Tachypnea	Jan. 23	Slight
85C00018	Twitching	Jan. 23	Moderate
	Clonic Convulsion	Jan. 23	Marked
	Inactive	Jan. 23	Moderate
	Tachypnea	Jan. 23,24	Moderate
	Tremors	Jan. 23	Moderate
	Depr. Grasping Reflex	Jan. 23	Marked
	Hunched Posture	Jan. 23	Slight
85C00020	Tachypnea	Jan. 23	Moderate
	Clonic Convulsion	Jan. 23	Moderate
	Irritable	Jan. 28,29	Slight
	Hyperactive	Jan. 28-Feb.6	Moderate
85C00024	Twitching	Jan. 23	Slight
	Clonic Convulsion	Jan. 23-29	Moderate
	Tachypnea	Jan. 23	Slight
	Hunched Posture	Jan. 23	Slight
	Inactive	Jan. 23	Slight
	Vocalization	Jan. 23	Slight
	Irritable	Jan. 23,28-Feb.6	Moderate
	Hyperactive	Jan. 28-Feb.6	Moderate
85C00043	Clonic Convulsion	Jan. 23	Moderate
	Inactive	Jan. 23	Slight
	Depr. Grasping Reflex	Jan. 23	Marked
	Hyperactive	Feb. 1,2	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 501 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00049	Clonic Convulsion	Jan. 23	Slight
	Tachypnea	Jan. 23	Slight
	Catalepsy	Jan. 23	Slight
	Depr. Grasping Reflex	Jan. 23	Slight
85C00052	Depr. Grasping Reflex	Jan. 23	Moderate
	Clonic Convulsion	Jan. 23	Moderate
	Hyperactive	Jan. 28-31	Slight
85C00060	Inactive	Jan. 23	Slight
	Incr. Respiration Rate	Jan. 23	Slight
	Tachypnea	Jan. 23	Moderate
	Clonic Convulsion	Jan. 23-Feb.6	Moderate
	Hyperactive	Feb. 3	Slight
	Stain, Yellow, Perianal	Feb. 4-6	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 562 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00001	Incr. Startle Reflex	Jan. 24	Slight
	Clonic Convulsion	Jan. 24	Marked
	Twitching	Jan. 24	Moderate
85C00002	Clonic Convulsion	Jan. 24	Marked
85C00005	Clonic Convulsion	Jan. 24	Slight
85C00006	Death	Jan. 24	1.0 h
85C00013	Clonic Convulsion	Jan. 24-27	Moderate
85C00014	Clonic Convulsion	Jan. 24	Slight
85C00037	Catalepsy	Jan. 24	Marked
	Inactive	Jan. 24	Moderate
	Tachypnea	Jan. 24	Moderate
	Incr. Startle Reflex	Jan. 24,28-29	Moderate
	Clonic Convulsion	Jan. 24	Marked
	Prostrate	Jan. 24	Slight
	Hyperactive	Feb. 1,2	Slight
85C00042	Clonic Convulsion	Jan. 24	Slight
85C00047	Misdosed	N/A	N/A
85C00056	Clonic Convulsion	Jan. 24	Marked
	Irritable	Jan. 25,30,31	Slight
	Hyperactive	Jan. 30,31	Moderate
85C00064	Tachypnea	Jan. 24	Moderate
	Inactive	Jan. 24	Slight
	Clonic Convulsion	Jan. 24	Marked
	Hunched Posture	Jan. 24	Slight
	Death	Jan. 24	2.0 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 631 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00004	Inactive	Jan. 23	Slight
	Depr. Grasping Reflex	Jan. 23	Slight
	Clonic Convulsion	Jan. 23	Marked
85C00010	Inactive	Jan. 23	Slight
	Tachypnea	Jan. 23	Moderate
	Clonic Convulsion	Jan. 23	Moderate
	Death	Jan. 23	1.2 h
85C00021	Inactive	Jan. 23	Slight
	Clonic Convulsion	Jan. 23	Moderate
	Depr. Grasping Reflex	Jan. 23	Slight
	Hunched Posture	Jan. 23	Slight
	Death	Jan. 23	5.0 h
85C00026	Death	Jan. 23	1.2 h
85C00028	Inactive	Jan. 23	Slight
	Clonic Convulsion	Jan. 23,24	Marked
	Depr. Grasping Reflex	Jan. 23	Slight
	Hunched Posture	Jan. 23	Slight
85C00032	Prostrate	Jan. 23	Marked
	Tremors	Jan. 23	Moderate
	Inactive	Jan. 23	Moderate
	Clonic Convulsion	Jan. 23	Marked
	Tachypnea	Jan. 23	Marked
	Tonic Convulsion	Jan. 23	Marked
	Depr. Grasping Reflex	Jan. 23	Marked
	Death	Jan. 23	1.2 h
85C00033	Inactive	Jan. 23	Marked
	Tachypnea	Jan. 23	Moderate
	Clonic Convulsion	Jan. 23	Marked
	Depr. Grasping Reflex	Jan. 23	Moderate
	Death	Jan. 23	1.0 h
85C00035	Depr. Grasping Reflex	Jan. 23	Slight
	Clonic Convulsion	Jan. 23	Moderate
	Death	Jan. 23	1.6 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 631 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00041	Inactive	Jan. 23	Marked
	Clonic Convulsion	Jan. 23	Moderate
	Depr. Grasping Reflex	Jan. 23	Slight
	Hunched Posture	Jan. 23	Slight
	Irritable	Jan. 23	Slight
	Vocalization	Jan. 23	Slight
	Hyperactive	Feb. 1,2	Slight
85C00063	Clonic Convulsion	Jan. 23	Slight
	Inactive	Jan. 23	Slight
	Death	Jan. 23	0.8 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 794 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00012	Depr. Grasping Reflex	Jan. 23	Slight
	Hyperactive	Jan. 23	Slight
	Vocalization	Jan. 23	Slight
	Clonic Convulsion	Jan. 23	Slight
	Hunched Posture	Jan. 23	Slight
	Death	Jan. 23	4.0 h
85C00019	Catalepsy	Jan. 23	Marked
	Inactive	Jan. 23	Moderate
	Hunched Posture	Jan. 23	Slight
	Clonic Convulsion	Jan. 23	Marked
	Incr. Respiration Rate	Jan. 23	Moderate
	Death	Jan. 23	0.8 h
85C00030	Inactive	Jan. 23	Moderate
	Hypertonia	Jan. 23	Moderate
	Twitching	Jan. 23	Slight
	Depr. Grasping Reflex	Jan. 23	Marked
	Jumping	Jan. 23	Slight
	Clonic Convulsion	Jan. 23	Slight
	Death	Jan. 23	0.8 h
85C00031	Depr. Grasping Reflex	Jan. 23	Slight
	Clonic Convulsion	Jan. 23	Moderate
	Tachypnea	Jan. 23	Moderate
	Inactive	Jan. 23	Slight
	Death	Jan. 23	4.0 h
85C00045	Inactive	Jan. 23	Slight
	Irritable	Jan. 23	Slight
	Clonic Convulsion	Jan. 23	Marked
	Depr. Grasping Reflex	Jan. 23	Slight
	Hunched Posture	Jan. 23	Moderate
	Vocalization	Jan. 23	Slight
	Tremors	Jan. 23	Marked
	Writhing	Jan. 23	Moderate

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 794 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00048	Clonic Convulsion	Jan. 23	Marked
	Inactive	Jan. 23	Slight
	Depr. Grasping Reflex	Jan. 23	Slight
	Death	Jan. 23	5.8 h
85C00050	Death	Jan. 23	0.6 h
85C00058	Depr. Grasping Reflex	Jan. 23	Moderate
	Hunched Posture	Jan. 23	Slight
	Clonic Convulsion	Jan. 23	Slight
	Irritable	Jan. 23	Slight
	Death	Jan. 23	3.4 h
85C00061	Clonic Convulsion	Jan. 23	Moderate
	Hunched Posture	Jan. 23	Slight
	Inactive	Jan. 23	Slight
	Depr. Grasping Reflex	Jan. 23	Slight
85C00062	Depr. Grasping Reflex	Jan. 23	Moderate
	Clonic Convulsion	Jan. 23	Moderate
	Hunched Posture	Jan. 23	Slight
	Inactive	Jan. 23	Slight
	Death	Jan. 24	18.8 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1000 mg/kg TMETN

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00011	Tremors	Jan. 25	Moderate
	Twitching	Jan. 25	Marked
	Tonic Convulsion	Jan. 25	Marked
	Hyperactive	Jan. 25	Moderate
	Catalepsy	Jan. 25	Slight
	Jumping	Jan. 25	Marked
	Death	Jan. 25	0.4 h
85C00016	Inactive	Jan. 25	Slight
	Catalepsy	Jan. 25	Slight
	Incr. Startle Reflex	Jan. 25	Marked
	Clonic Convulsion	Jan. 25	Moderate
	Tremors	Jan. 25	Moderate
	Death	Jan. 25	4.6 h
85C0023	Incr. Startle Reflex	Jan. 25	Marked
	Tachypnea	Jan. 25	Moderate
	Hunched Posture	Jan. 25	Moderate
	Inactive	Jan. 25	Slight
	Twitching	Jan. 25	Slight
	Clonic Convulsion	Jan. 25	Marked
	Death	Jan. 25	1.9 h
85C00027	Inactive	Jan. 25	Slight
	Incr. Startle Reflex	Jan. 25	Moderate
	Clonic Convulsion	Jan. 25	Marked
	Catalepsy	Jan. 25	Slight
	Death	Jan. 25	2.0 h
85C00029	Incr. Startle Reflex	Jan. 25	Marked
	Jumping	Jan. 25	Moderate
	Clonic Convulsion	Jan. 25	Moderate
	Twitching	Jan. 25	Moderate
	Catalepsy	Jan. 25	Moderate
	Hunched Posture	Jan. 25	Moderate
	Tachypnea	Jan. 25	Moderate
	Depr. Grasping Reflex	Jan. 25	Marked
	Death	Jan. 25	2.0 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1000 mg/kg TMETN (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00034	Twitching	Jan. 25	Marked
	Tremors	Jan. 25	Marked
	Tonic Convulsion	Jan. 25	Marked
	Catalepsy	Jan. 25	Moderate
	Inactive	Jan. 25	Slight
	Incr. Startle Reflex	Jan. 25	Moderate
	Depr. Righting Reflex	Jan. 25	Slight
	Depr. Grasping Reflex	Jan. 25	Marked
	Death	Jan. 25	0.6 h
85C00038	Incr. Startle Reflex	Jan. 25	Slight
	Depr. Grasping Reflex	Jan. 25	Moderate
	Clonic Convulsion	Jan. 25-29	Marked
	Hyperactive	Jan. 28-Feb.6	Moderate
85C00039	Clonic Convulsion	Jan. 25	Moderate
	Incr. Startle Reflex	Jan. 25	Slight
	Tachypnea	Jan. 25	Moderate
	Death	Jan. 25	2.5 h
85C00044	Clonic Convulsion	Jan. 25	Marked
	Irritable	Jan. 25	Slight
	Incr. Startle Reflex	Jan. 25	Marked
	Catalepsy	Jan. 25	Slight
	Death	Jan. 25	2.2 h
85C00059	Death	Jan. 25	0.3 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: Vehicle Controls

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00015	Normal	N/A	N/A
85C00025	Feces, Perianal	Jan. 23	Slight
	Irritable	Jan. 25	Slight
85C00040	Misdosed	N/A	N/A
85C00053	Depr. Grasping Reflex	Jan. 23	Slight
85C00055	Irritable	Jan. 23,30	Slight
	Hyperactive	Jan. 30	Moderate

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: Cage Control

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00022	Normal	N/A	N/A
85C00046	Vocalization	Jan. 23	Slight
	Irritable	Jan. 23	Slight
85C00051	Normal	N/A	N/A
85C00054	Normal	N/A	N/A
85C00057	Inactive	Jan. 23	Slight
	Depr. Grasping Reflex	Jan. 28,29	Moderate

Appendix F: INDIVIDUAL BODY WEIGHTS (g)

Males: 631 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
84C00507	25	29	31	33
84C00512	24	31	35	36
84C00519	26	30	31	32
84C00520	24	27	22	25
84C00527	23	28	31	33
84C00536	24	28	31	33
84C00552	30	33	35	35
84C00559	25	29	34	34
84C00560	26	29	30	32
84C00561	27	31	Dead	
Mean	25.4	29.5	31.1	32.6
Standard Deviation	2.0	1.8	3.9	3.1
Standard Error of the Means	0.6	0.6	1.3	1.0

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS (g)

Males: 794 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
84C00506	28	32	34	36
84C00518	26	29	33	35
84C00522	24	28	Dead	
84C00523	25	29	32	33
84C00525	26	30	Dead	
84C00541	25	28	Dead	
84C00542	27	29	32	33
84C00544	28	31	33	36
84C00562	28	31	28	30
84C00563	24	28	Dead	
Mean	26.1	29.5	32.0	33.8
Standard Deviation	1.6	1.4	2.1	2.3
Standard Error of the Mean	0.5	0.4	0.9	0.9

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS (g)

Males: 1000 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
84C00511	24	28	Dead	
84C00530	25	27	Dead	
84C00533	28	31	Dead	
84C00537	25	29	Dead	
84C00546	23	28	Dead	
84C00548	27	29	Dead	
84C00551	26	32	35	36
84C00553	25	29	Dead	
84C00556	29	35	Dead	
84C00558	26	31	34	35
Mean	25.8	29.9	34.5	35.5
Standard Deviation	1.8	2.4	0.7	0.7
Standard Error of the Means	0.6	0.8	0.5	0.5

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS (g)

Males: 1260 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
84C00514	27	30	Dead	
84C00531	28	30	Dead	
84C00534	23	26	Dead	
84C00535	Removed from study			
84C00540	25	28	Dead	
84C00543	25	28	Dead	
84C00547	25	28	Dead	
84C00550	25	29	Dead	
84C00555	28	32	Dead	
84C00557	27	31	Dead	
Mean	25.9	29.1		
Standard Deviation	1.7	1.8		
Standard Error of the Mean	0.6	0.6		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS (g)

Males: 1590 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
84C00513	25	30	Dead	
84C00515	29	30	Dead	
84C00517	29	25	Dead	
84C00521	25	30	Dead	
84C00526	31	35	Dead	
84C00529	25	30	Dead	
84C00538	25	30	Dead	
84C00539	24	28	Dead	
84C00545	26	32	Dead	
84C00554	26	29	Dead	
Mean	26.5	29.9		
Standard Deviation	2.3	2.6		
Standard Error of the Mean	0.7	0.8		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS (g)

Females: 501 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00003	29	28	29	29
85C00007	28	29	31	32
85C00009	23	26	29	30
85C00018	28	27	28	30
85C00020	26	26	26	27
85C00024	25	26	27	28
85C00043	26	28	30	31
85C00049	28	28	29	30
85C00052	22	26	26	29
85C00060	26	29	31	31
Mean	26.1	27.3	28.6	29.7
Standard Deviation	2.3	1.3	1.8	1.5
Standard Error of the Mean	0.7	0.4	0.6	0.5

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS (g)

Females: 562 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00001	28	29	31	31
85C00002	27	30	32	32
85C00005	28	31	32	32
85C00006	29	29	Dead	
85C00013	28	29	30	30
85C00014	27	26	27	27
85C00037	27	26	21	25
85C00042	25	25	26	26
85C00056	27	29	30	29
85C00064	29	29	Dead	
Mean	27.5	28.3	28.6	29.0
Standard Deviation	1.2	1.9	3.8	2.7
Standard Error or the Mean	0.4	0.6	1.3	1.0

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS (g)

Females: 631 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00004	28	28	28	29
85C00010	26	29	Dead	
85C00021	25	26	Dead	
85C00026	27	27	Dead	
85C00028	28	27	28	29
85C00032	29	32	Dead	
85C00033	31	29	Dead	
85C00035	25	25	Dead	
85C00041	28	29	32	32
85C00063	27	27	Dead	
Mean	27.4	27.9	29.3	30.0
Standard Deviation	1.8	2.0	2.3	1.7
Standard Error of the Mean	0.6	0.6	1.3	1.0

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS (g)

Females: 794 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00012	29	27	Dead	
85C00019	27	27	Dead	
85C00030	26	27	Dead	
85C00031	28	30	Dead	
85C00045	26	27	30	31
85C00048	23	25	Dead	
85C00050	27	28	Dead	
85C00058	32	32	Dead	
85C00061	27	25	27	28
85C00062	26	27	Dead	
Mean	27.1	27.5	28.5	29.5
Standard Deviation	2.3	2.1	2.1	2.1
Standard Error of the Mean	0.7	0.7	1.5	1.5

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS (g)

Females: 1000 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00011	28	28	Dead	
85C00016	25	30	Dead	
85C00023	27	28	Dead	
85C00027	26	26	Dead	
85C00029	25	24	Dead	
85C00034	29	29	Dead	
85C00038	27	28	26	26
85C00039	24	27	Dead	
85C00044	29	30	Dead	
85C00059	28	30	Dead	
Mean	26.8	28.0		
Standard Deviation	1.8	1.9		
Standard Error of the Mean	0.6	0.6		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS (g)

Vehicle Control

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
		Males		
84C00508	27	29	32	33
84C00510	25	29	31	33
84C00516	25	30	31	33
84C00524	27	30	33	34
84C00532	28	31	33	35
Mean	26.4	29.8	32.0	33.6
Standard Deviation	1.3	0.8	1.0	0.9
Standard Error of the Mean	0.6	0.4	0.4	0.4
		Females		
85C00015	23	27	28	27
85C00025	27	27	29	30
85C00040	26	27	Misdosed	
85C00053	28	28	29	28
85C00055	26	27	26	27
Mean	26.0	27.2	28.0	28.0
Standard Deviation	1.9	0.4	1.4	1.4
Standard Error of the Mean	0.8	0.2	0.7	0.7

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS (g)

Cage Control

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
		Females		
85C00022	28	30	29	30
85C00046	26	28	27	26
85C00051	28	30	30	29
85C00054	27	28	28	29
85C00057	26	29	29	29
Mean	27.0	29.0	28.6	28.6
Standard Deviation	1.0	1.0	1.1	1.5
Standard Error of the Mean	0.4	0.4	0.5	0.6

Appendix G: PATHOLOGY REPORT

Oral Lethal Dose (MLD) Test in Mice of 1, 3 - Propanediol, 2-(hydroxymethyl)-2-methyl-, trinitrate (TMETN)

History: This study was designed to determine the oral toxicity of TMETN in male and female mice. CD-1 (ICR) mice, average weight 30 grams, were divided into thirteen (13) groups with varying numbers per group (see table). After accummulation and randomization, these animals were dosed by oral gavage as follows:

MALES

Group 1 - 10 ml/kg vehicle control (corn oil)
Group 2 - 631 mg/kg TMETN
Group 3 - 794 mg/kg TMETN
Group 4 - 1000 mg/kg TMETN
Group 5 - 1260 mg/kg TMETN
Group 6 - 1590 mg/kg TMETN

FEMALES

Group 1 - cage control
Group 2 - 10 ml/kg vehicle control (corn oil)
Group 3 - 562 mg/kg TMETN
Group 4 - 501 mg/kg TMETN
Group 5 - 631 mg/kg TMETN
Group 6 - 794 mg/kg TMETN
Group 7 - 1000 mg/kg TMETN

Microscopic Findings:

Pathology Acc. No.	Animal ID No.	Morphologic Diagnosis
36408	84C00524	Hepatitis,necrotizing, acute, multifocal, moderate liver.

Gross Necropsy Findings:

Thirty-two of the 49 males exposed to TMETN died within the first 24-hour observation period. Two mice that died, one from group 3 and one from group 5 had excessive red staining of the muzzle. One mouse from group 4 had a healing femoral fracture. One mouse from the vehicle control group had multifocal 1 mm diameter slightly raised white foci on the serosal surface of the liver. The remaining male mice had no gross lesions at necrospy.

Appendix G (cont.): PATHOLOGY REPORT

Females - Twenty-six of the 50 females exposed to TMETN died within the first 24-hour observation period. No gross lesions were observed at necropsy in the female groups.

TABLE 1 - MALES

Group No.	1	2	3	4	5	6
Animals/Group	5	10	10	10	9	10
Deaths	0	1	4	8	9	10
Lesions	1	0	1	1	1	
Liver - multiple white foci	1					
Muzzle - red staining			1		1	
Femur - healing fracture				1		

TABLE 2 - FEMALES

Group No	1	2	3	4	5	6	7
Animals/Group	5	4	10	10	10	10	10
Deaths	0	0	2	0	7	8	9

Summary:

1. Dose related toxicity of the compound is apparent. The difference noted between the males and females is possibly due to sexual dimorphism.
2. Red staining of the muzzle of a couple of male mice was most likely agonal hemorrhage from the nasal passages and/or lacrimal gland discharge through the lacrimal nasal ducts at the time of death. The femoral fracture was an incidental unrelated finding. The cause of the hepatitis in the male vehicle control mouse cannot be determined. Special stains were noncontributory.
3. One female from group 3 was misdosed. It was removed from the study and submitted for necropsy. No lesions were recognized.
4. One mouse from group 5 (males) was actually a female (ID No. 84C00535, LAIR Accession No. 36354). It was removed from the study.

G. TRACY MAKOVEC, DVM
CPT, VC
Pathology Services Group

LANCE O. LOLLINI, DVM
LTC, VC
Chief, Pathology Services Group

Distribution List

Commander
US Army Biomedical Research and Development Laboratory (15)
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center (DTIC) (2)
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and Development Command (2)
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commander US Army Materiel Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander
US Army Environmental Hygiene Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

Dean
School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

Commander
US Army Materiel Command
ATTN: AMCEN-A
5001 Eisenhower Avenue
Alexandria, VA 22333

HQDA
ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
2C Massachusetts, NW
Washington, D.C. 20314

CDR, US Army Toxic and Hazardous Material Agency
ATTN: DRXTH/ES
Aberdeen Proving Ground, MD 21010

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
Preventive Medicine Division (HSHA-IPM)
Fort Sam Houston, TX 78234